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Potential role of MG53 in the regulation of transforming-growth-factor-β1-induced atrial fibrosis and vulnerability to atrial fibrillation

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ABSTRACT

Atrial fibrosis plays a critical role in atrial fibrillation (AF) by the transforming growth factor (TGF)-β1/Smad pathway. The disordered differentiation, proliferation, migration and collagen deposition of atrial fibroblasts play significant roles in atrial fibrosis. Mitsugumin (MG)53 is predominantly expressed in myocardium of rodents and has multiple biological functions. However, the role of MG53 in cardiac fibrosis remains unclear. This study provided clinical and experimental evidence for the involvement of MG53 in atrial fibrosis in humans and atrial fibrosis phenotype in cultured rat atrial fibroblasts. In atrial tissue from patients we demonstrated that MG53 was expressed in human atrium. Expression of MG53 increased with the extent of atrial fibrosis, which could induce AF. In cultured atrial fibroblasts, depletion of MG53 by siRNA caused down-regulation of the TGF-β1/Smad pathway, while overexpression of MG53 by adenovirus up-regulated the pathway. MG53 regulated the proliferation and migration of atrial fibroblasts. Besides, exogenous TGF-β1 suppressed expression of MG53. In conclusion, we demonstrated that MG53 was expressed in human atrium, and may be a potential upstream of the TGF-β1/Smad pathway in human atrium and rat atrial fibroblasts. This suggests that MG53 is a potential regulator of atrial fibrosis induced by the TGF-β1/Smad pathway in patients with AF.

1. Introduction

Atrial fibrillation (AF) is one of the most common arrhythmias and its prevalence increases with age [1–3]. Electrical remodeling and structural remodeling are major mechanisms that contribute to AF. While electrical remodeling is reversible, structural remodeling remains fundamentally related to the development and maintenance of AF [3–6]. Atrial fibrosis, the hallmark of structural remodeling, is identified as a substrate of AF [7,8], and is considered to be a potential therapeutic target in the treatment of AF [9]. Atrial fibroblasts are the main determinant of atrial fibrosis [9,10]. The disordered differentiation, migration, proliferation and extracellular matrix (ECM) synthesis of atrial fibroblasts is considered to be pivotal causes of atrial fibrosis [9,11].

The transforming growth factor (TGF) -β1/Smad pathway plays a critical role in matrix remodeling, capable of activating atrial fibroblasts differentiation and promoting cellular migration, proliferation and ECM synthesis, which are associated with atrial fibrosis and AF [12–16]. Several studies have demonstrated that TGF-β1 had a profibrotic effect via Smad proteins, which could potentiate atrial fibrosis and AF [15–17]. Our own studies have implicated that, in human atrial tissues, the TGF-β1/Smad pathway was activated in the atrium of patients with rheumatic heart disease (RHD) with AF, compared to those with RHD with sinus rhythm (SR) and those with congenital heart disease (CHD) and so did atrial fibrosis extend [3,17]. In cultured rat atrial fibroblasts, TGF-β1 stimulation is sufficient to generate atrial fibrosis phenotype [18].

Mitsugumin (MG)53, also named TRIM72, is a muscle-specific tripartite motif family protein of the E3 ubiquitin ligases [19]. MG53 is also an essential component of the acute membrane repair process by nucleating the assembly of the repair machinery at injury sites [19]. MG53 is mainly expressed in skeletal and cardiac muscle of rodents and shows cardioprotective effects in animal models via cell membrane repair [20,21]. In addition, MG53 acts as an E3 ligase to mediate the degradation of insulin receptor and insulin receptor substrate-1, which subsequently induces insulin resistance and metabolic diseases

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[22–24]. MG53 is a potential therapeutic target in human diseases and has multiple biological functions, as a double-edged sword for human diseases [25]. However, the biological function and mechanisms of MG53 in the TGF-β1 signaling pathway and cardiac fibrosis remain elusive.

In the present study, we researched on human atrial tissue and rat atrial fibroblasts to investigate the relationship between MG53 and the TGF-β1 signaling pathway, and the role of MG53 in atrial fibrosis and AF.

2. Material and methods

2.1. Patients and specimens

We enrolled 30 patients (19 female and 11 male; mean age 43.8 ± 9.8 years) who consecutively underwent corrective heart surgery between April and December 2016 in the First Affiliated Hospital of Chongqing Medical University. The patients were divided into three groups: CHD + SR (n = 10)(used as controls), RHD + SR (n = 10), and RHD + AF (n = 10). Electrocardiograms, chest X-rays and echocardiograms were recorded. Patients had New York Heart Association (NYHA) class II–III heart function. Patients with hypertension, coronary heart disease, diabetes mellitus, cardiomyopathy, hyperthyroidism, malignant tumor, or patients aged > 70 years were excluded. Patients stopped use angiotensin-converting enzyme inhibitors or angiotensin receptor blockers for at least six months before surgery.

Tissue samples of right atrial appendages (> 100 mg) were obtained before the establishment of extracorporeal circulation. Samples were divided into two parts: one was fixed in 4% paraformaldehyde for histological staining and immunohistochemistry; and the other was flash frozen in liquid nitrogen and stored at −80 °C for quantitative real-time polymerase chain reaction (PCR) and western blotting.

All human studies abode strictly by the principles of the Declaration of Helsinki. All patients or their family members provided written informed consent. The study was conducted under permission of the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University. The clinical data of patients are summarized in Table 1.

2.2. Histological staining and fibrosis quantification

Samples were fixed in 4% paraformaldehyde and embedded in paraffin. Four-micrometer-thick sections were cut and stained with Masson's trichrome. Each sample was observed randomly in five different fields under an Olympus BX51 microscope (Tokyo, Japan) at 400× magnification. The captured images were analyzed by ImagePro Plus 6.0 image analysis system. Collagen volume fraction (CVF) was presented as the area ratio of collagen fiber and the whole image.

Table 1
Clinical data of patients.

	CHD + SR	RHD + SR	RHD + AF
Number of cases	10	10	10
Gender, M/F	4/6	3/7	4/6
Age, years	36.1 ± 10.5	48.7 ± 8.5	46.2 ± 5.4
LVEF, %	67.0 ± 1.2	64.3 ± 2.6	59.7 ± 6.4
LAD, mm	25.8 ± 1.5	36.3 ± 4.6*	47.0 ± 9.3*#
LVEDD, mm	41.8 ± 5.1	47.0 ± 6.0	48.5 ± 7.3
RAD, mm	39.6 ± 7.2	33.0 ± 3.4	37.8 ± 4.3

Data are expressed as mean ± SEM. LVEF = left ventricular ejection fraction; LAD = left atrial dimension; LVEDD = left ventricular end-diastolic dimension; RAD = right atrial dimension.

* $p < 0.001$ compared with CHD + SR.

# $p < 0.05$ compared with RHD + SR.

2.3. Immunohistochemical studies

Immunohistochemical staining was performed using an immunohistochemistry kit obtained from Zhong Shan Golden Bridge Biotechnology Corp. (Beijing, China). Four-micrometer-thick sections were deparaffinized and washed with phosphate-buffered saline (PBS) and blocked with 1% goat serum albumin in PBS for 30 min. Sections were incubated with a rabbit anti-TGF-β1 (1:100; Abcam, UK) and rabbit anti-MG53 (1:100; provided by J.M.) antibody overnight at 4 °C. After incubation, the sections were washed with PBS, and incubated with the peroxidase-conjugated goat anti-rabbit immunoglobulin G for 30 min. The sections were washed with PBS again, and the diaminobenzidine (DAB) was applied to detect peroxidase activity. Sections were counterstained with hematoxylin, dehydrated and mounted with neutral balsam. Images were captured by Olympus BX51 microscope (Tokyo, Japan) at 200× magnification and analyzed by ImagePro Plus 6.0 image analysis system. The levels of the MG53 and TGF-β1 were determined with Integrated option density (IOD).

2.4. Cardiac fibroblast isolation and cell culture

Atrial fibroblasts derived from atrial tissue pieces from 14-day-old neonatal Sprague–Dawley rats and identified by immunofluorescence as described previously [18]. Rat atrium was removed using small scissors and forceps, then cut into 1–2-mm^2 segments and minced in a six-well plate. The cells were cultured in Dulbecco's modified Eagle's medium: Nutrient Mixture F-12 (Gibco, CA, USA) containing 15% fetal bovine serum (Wisent, MTL, CA) and 1% penicillin–streptomycin–Fungizone and incubated with 5% CO_2 at 37 °C. The medium was changed at 2 - day intervals. Cells at passages 3–5 were used for the experiment. These procedures were approved by the Ethics Committee of Chongqing Medical University Animal Subject Committee.

2.5. RNA interference

The sequences of siRNA (RiboBio, Guangzhou, China) used to knock down expression of tripartite motif - containing protein (Trim) 72 were as follows: Trim72 - si – 1RNA, 5′-GCAAGAUCCUGGAGGCACATT-3′; Trim72 - si – 2RNA, 5′ - AGGCACACGUGGAGGCCAATT – 3′, and Trim72 - si – 3RNA, 5′ - AACUGAGGCAGAUGGAGAATT – 3′. The negative control (NC) siRNA was provided by RiboBio. Cells were cultured in six-well plates (8×10^4 cells/well) without antibiotics, and 60% confluence was used as a sign of transfection. 100 pmol siRNA oligomer was delivered into each well with riboFECT™ CP Buffer and riboFECT™ CP Reagent (RiboBio). Cells without transfection were used as controls. The cells were incubated at 37 °C in a CO_2 incubator for 24–96 h until they were used for the assay for gene knockdown.

2.6. Adenovirus vector

The recombinant adenovirus vectors of MG53 (Ad-MG53) and green fluorescent protein (Ad-GFP) were purchased from Genemine (Chongqing, China). Cells were seeded in a six-well plate (10^5 cells/well). After incubation at 37 °C overnight, the recombinant adenovirus vector diluted in culture medium with appropriate multiplicity of infection was added to the cells. Cells without transfection were used as controls. The medium was changed after overnight incubation, and then incubated at 37 °C for 24–96 h until used for the assay for gene overexpression.

2.7. Migration assays

Transwell Boyden chambers (24-well insert, 8.0-mm; BD Biosciences, NJ, USA) were used to detected the migration of atrial fibroblasts. After transfection for 24 h, 10^4 cells of each group were seeded in the upper chamber with the uncoated membranes in the